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OBSERVATIONS ON THE VIRUS AND MEANS OF TRANSMISSION OF ROCKY MOUNTAIN SPOTTED FEVER.*†

H. T. RICKETTS, M.D.
Instructor in Pathology, University of Chicago.

(From the Pathological Laboratory of the University of Chicago.)

INVESTIGATIONS into the nature of Rocky Mountain Spotted Fever its etiology and method of transmission, have been carried on by the writer since April, 1906.

Previous to these investigations the disease had been studied extensively by Wilson and Chowning,¹ and to some degree also by members of the Public Health and Marine Hospital Service. I shall refer to these investigations only to say that Wilson and Chowning described the disease as a pyroplasmosis, and advanced the important theory that man is infected by the bite of the "wood-tick" which infests the mountainous regions of Montana and adjacent Rocky Mountain States. They furnished no experimental proof of the correctness of the theory. Following the report of Wilson and Chowning, Stiles,² of the Public Health and Marine Hospital Service, studied the disease and failed utterly to find the pyroplasma of the former investigators. Stiles discredited also the theory of transmission by the tick, but without experimental evidence to refute the theory.

This was the status in relation to the etiology and means of transmission of the disease when my studies were undertaken. The results which I have obtained have been described briefly in three communications to the *Journal of the American Medical Association*.³

The essential points presented in these articles are the following:

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¹ *Jour. Amer. Med. Assoc.*, 1902, 39, p. 131; also *Jour. Infect. Dis.*, 1904, 1, p. 31.

² *Pub. Health and Mar. Hosp. Bull.*, No. 20, 1905.

³ (1) "The Study of 'Rocky Mountain Spotted Fever' (Tick Fever) by means of Animal Inoculations," *Jour. Am. Med. Assoc.*, July 7, 1906, 47, pp. 33-36; (2) "The Transmission of Rocky Mountain Spotted Fever by the Bite of the Wood-Tick (*Dermacentor occidentalis*)," *ibid.*, August 4, 1906, 47, p. 358; (3) "Further Observations on Rocky Mountain Spotted Fever and *Dermacentor occidentalis*," *ibid.*, October 6, 1906, 47, pp. 1067-69.

The disease was transmitted to guinea-pigs from three different cases, and to monkeys from two different cases by inoculation with defibrinated blood from the patients (1, 3). From the last of the three cases the disease has been maintained in the laboratory by alternate passage through the monkey and the guinea-pig, by means of inoculations with blood or emulsions of organs (3). The first attempts to maintain the infection by direct inoculation from guinea-pig to guinea-pig failed, possibly for reasons which will be referred to below. Rabbits were found not to be susceptible to an appreciable degree, and the same was true of white mice and white rats. In a preliminary experiment the virus did not pass through a Berkefeld filter, although the unfiltered serum was infectious. The anatomical similarity of the disease produced in the guinea-pig and monkey to the conditions observed in man, and the incubation period and course of fever induced by inoculation, were considered sufficient evidence of the genuine transmission of the disease to these animals (1, 3). In a single experiment a tick, female, was found to be the carrier of the disease from one guinea-pig to another. This result was considered as tentative (2).¹

The virus was found to be distributed in all of the visceral organs, the most vascular organs (liver, spleen, and bone marrow) and the kidney apparently being the most infectious (3).

It was found that an active immunity which possibly is relative in degree, is established in the monkey and guinea-pig by one attack (3). The life-history of *Dermacentor occidentalis* was followed under laboratory conditions. This tick passes through a larval stage and moults twice subsequently, before reaching the adult form, the cycle from egg to adult requiring about three months under the modified conditions, which prevailed (3). The tick left the host in order to moult.

It is the purpose of this paper to present further observations which have been made in relation to the distribution of the virus in the body fluids, its viability and resistance under different conditions, its filterability and certain other properties, the possibility of transmission by means of the bite of the male tick, and in addition, the preservation of the virus by uninterrupted passage through the guinea-pig.

¹ King also has reported transmission by means of the female tick (*Pub. Health Reports*, . . .).

THE DISTRIBUTION OF THE VIRUS IN THE BLOOD.

Rocky Mountain Spotted Fever is unquestionably a systemic infection, since the disease may be transmitted by the inoculation with blood and with emulsions of the solid organs.

A study of the distribution of the virus in the blood was undertaken with the hope that the question of an exclusive or predominant erythrocytic infection, such as a pyroplasmosis, might be affirmatively or negatively determined. Although the experiments reported are not quantitative in character, it is thought that the sum total of results renders the pyroplasma theory open to suspicion. Quantitative work is very difficult from the fact that the concentration of the virus in different animals is subject to considerable variation; this may be as great as a tenfold variation, the minimum fatal dose of defibrinated blood in one instance being 0.05 c.c. and in another 0.5 c.c.

The following results have been obtained in regard to this phase of the work:

1. It is impossible to free the blood cells of defibrinated blood from the virus by 10 to 12 washings with physiologic salt solution (Table 3). Repeated washing, however, seems to decrease the virulence of the corpuscles of infected blood (Table 7).
3. The serum obtained from defibrinated blood or from spontaneous clotting is infectious in doses of 0.5 c.c. or even less (Table 5).
4. Prolonged centrifugation of the serum (6 hours) does not free the overlying portion from the virus (Table 8).
5. An exudate of leucocytes in the pleural or peritoneal cavity of the infected monkey or guinea-pig, caused by the injection of aleuronat or bouillon, is infectious for the guinea-pig. The sediment of such an exudate is not deprived of its infectiousness by repeated washings, and the overlying fluid remains infectious in spite of prolonged centrifugation (Table 6).
2. As washing advances a point is reached at which the virus is not separated from the corpuscles in infective quantities by shaking with salt solution. An experiment showed that a fluid of the 10th washing was not infectious in quantities of 5 and 10 c.c., whereas the cellular sediment caused the disease in doses of 0.7 and 1.5 c.c. (Tables 3 and 4).

The following selected experiments are given as illustrative of the preceding statements:

TABLE 1.

CONTROL EXPERIMENT. INOCULATIONS WITH FRESH UNDEFIBRINATED BLOOD OF MONKEY XIV.

| Guinea-Pig No. | Dose | Incubation Period | Duration from Date of Inoculation | Result | Autopsy |
|----------------|-----------|-------------------|-----------------------------------|--------|----------|
| 150 | 0.05 c.c. | 3-4 days | 12 days | Death | Typical* |
| 151 | 0.1 | 3-4 | 10½ | " | " |
| 152 | 0.3 | 3-4 | 11 | " | " |
| 153 | 0.7 | 3-4 | 9 | " | " |

* The following conditions at autopsy are recognized as typical for spotted fever in the guinea-pig: Generalized enlargement of the lymph glands including those of the mesentery and mediastinum. Either extreme congestion of, or hemorrhage into, the lymph glands. Enlargement of the spleen, which may reach the size of two or four times the mass of the normal spleen. The spleen is much congested and cyanotic, fairly homogeneous and of moderate consistency, i. e., neither soft nor hard. The kidneys suprarenal glands, and liver are somewhat enlarged and congested. The right heart and veins are heavily engorged. The lungs show no changes. The meninges are moderately reddened. The bone marrow is rich in rather dark red blood. Very frequent, but not constant changes are hemorrhages into the skin of the external genitalia, and, in males, into the testicles and their coverings. Hemorrhage also occurs with some frequency into the suprarenal gland, and less often, into the liver. If visible colonies appear on a plate of the heart's blood which is made soon after death, or if peritonitis or other severe local inflammation is found, the animal has been disregarded.

TABLE 2.

CONTROL EXPERIMENT. INOCULATIONS WITH FRESH DEFIBRINATED BLOOD OF MONKEY XIV.

| Guinea-Pig No. | Dose | Incubation Period | Duration from Date of Inoculation | Result | Autopsy |
|----------------|-----------|-------------------|-----------------------------------|--------|---------|
| 154 | 0.05 c.c. | 5-6 days | 13 days | Death | Typical |
| 155 | 0.1 | 5-6 | 11 | " | " |
| 156 | 0.3 | 3-4 | 12 | " | " |
| 157 | 0.7 | 3-4 | 11 | " | " |

TABLE 3.

INOCULATIONS WITH WASHED DEFIBRINATED BLOOD OF MONKEY XIV: TEN WASHINGS, THE ORIGINAL VOLUME BEING RESTORED.

| Guinea-Pig No. | Dose | Incubation Period | Duration from Date of Inoculation | Result | Autopsy |
|----------------|----------|-------------------|-----------------------------------|----------|---------|
| 162† | 0.3 c.c. | 5 days | 8 days | Not sick | Typical |
| 163 | 0.7 | | | Killed | |
| 164 | 1.0 | | | Not sick | |
| 165 | 1.5 | 5-6 | 13 | Death | " |

†Not sick. Died later, following rectal prolapse.

TABLE 4.

INOCULATIONS WITH OVERLYING SALT SOLUTION OF THE TENTH WASHING OF BLOOD USED IN TABLE 3.

| No. | Dose | Result |
|-----------|---------|----------|
| 167 | 10 c.c. | Not sick |

TABLE 5.

INOCULATIONS WITH SERUM FROM BLOOD OF MONKEY XIV, THE SERUM BEING OBTAINED BY DEFIBRINATION AND CENTRIFUGATION FOR ONE HOUR.

| Guinea-Pig No. | Dose | Incubation Period | Duration from Date of Inoculation | Result | Autopsy |
|----------------|------|-------------------|-----------------------------------|--------|---------|
| 158 | 1.0 | 3-4 days | 11 days | Death | Typical |
| 159 | 2.0 | 3-4 | 13 | " | " |
| 160 † | 3.0 | | | | |
| 161 | 4.0 | 3-4 | 14 | " | " |

† Accidental death in 4 days.

TABLE 6.

INFECTIOUS PROPERTIES OF LEUCOCYTIC EXUDATE FROM MONKEY XIV, CAUSED BY AN INJECTION OF ALEURONAT.

| Guinea-Pig No. | Dose and Material Inoculated | Incubation Period | Duration from Date of Inoculation | Result | Autopsy |
|----------------|--|-------------------|-----------------------------------|----------|---------|
| 147. | 2 c.c. of unaltered exudate | 6 days | 13 days | Death | Typical |
| 148. | 5 c.c. of leucocyte suspension, washed 3 times | 6 | 14 | " | " |
| 149. | 4½ c.c. of fluid from first centrifugation | 6-7 | 21 | Recovery | |

TABLE 7.

THE EFFECT OF REPEATED WASHING OF BLOOD IN DECREASING ITS VIRULENCE. MIXED BLOODS OF MONKEYS VII AND VIII 12 C. C. INJECTED INTO EACH GUINEA-PIG.

| Guinea-Pig No. | Washings | Incubation Period | Duration from Date of Inoculation | Result | Autopsy |
|----------------|----------|-------------------|-----------------------------------|--------|---------|
| 44..... | 1 | 2 days | 7 days | Death | Typical |
| 45..... | 2 | 4 | 10 | " | " |
| 47..... | 5 | 5 | 14 | " | " |
| 49..... | 10 | 5 | 16 | " | " |

TABLE 8.

ATTEMPT TO REMOVE INFECTIOUS PROPERTIES OF SERUM BY CENTRIFUGATION FOR SIX HOURS AT THE RATE OF 2,000 REVOLUTIONS PER MINUTE. 5 C.C. OF SERUM FROM MONKEYS VII AND VIII

| Guinea-Pig No. | Injection | Incubation Period | Duration | Result |
|----------------|-------------------------|-------------------|----------|----------|
| 50..... | Centrifugation serum | 7-8 days | 20 days | Recovery |
| 51..... | Final 0.5 c.c. of above | 5 | 16 | " |

The tenacity with which the virus associates itself with the blood corpuscles suggests that a certain proportion of the parasites may be within either the leucocytes or the erythrocytes. This suggestion seems the stronger since, as shown in Guinea-pig 167 (Table 4), a certain proportion of the virus is not readily separated from the corpuscles by moderate agitation with salt solution, but remains

rather firmly associated with the blood cells (Table 3). In relation to this fact, however, the following points are to be observed: First, the density and viscosity of serum is considerably greater than that of 0.85 per cent salt solution, and although the virus cannot be separated from serum readily by centrifugation, such separation could be more readily accomplished in salt solution. Hence it is still possible that the absence of the virus in pathogenic quantity from the salt solution of the 10th washing may be caused in large part by the sedimentation necessary for the separation of the corpuscles. Not only must the difference in the density of serum and salt solution cause a wide difference in the ease with which microbes may be sedimented from their solutions, but the coarse physical structure of serum must interfere greatly with the sedimentation of minute particles. In the second place it is readily conceivable that minute organisms may adhere to the external surface of erythrocytes and leucocytes so firmly that moderate agitation in salt solution does not cause their separation in pathogenic quantities.

In an experiment, the protocol of which is not given, 0.5 c.c. of serum obtained after spontaneous clotting caused fatal infection in the guinea-pig. This would suggest a general plasmatic infection rather than one which is essentially cellular.

Since a leucocytic exudate practically devoid of erythrocytes is infectious, it seems probable that the condition could not well be considered as an essential involvement of the erythrocytes. Furthermore, from the fact that the virus exists in the fluid portion of the leucocytic exudate in pathogenic quantities, the infection could hardly be considered as essentially leucocytic in character.

Although it is desirable to wait until certain quantitative experiments are completed before drawing positive conclusions as to the situation of the virus, I believe the qualitative results described above indicate a general plasmatic infection rather than an essential invasion of blood cells.

FILTRATION AND INTOXICATION.

Filtration experiments have been continued with variations in conditions. Small Berkefeld candles have been used exclusively and for the most part those which were fresh from the factory. In case a

filter had been previously used, it was washed with distilled water in both directions, subjected to prolonged boiling in a solution of sodium carbonate, and washed first with salt solution and then with distilled water in both directions, before being used again. It has seemed useless to employ porcelain filters so long as the virus has not been passed through the more porous Berkefeld filters.

No greater pressure has been used than that obtained from a vacuum water pump, with a pressure of from 30 to 40 pounds in the mains. In all experiments except one, the filters have been kept covered with the serum by drawing the latter into a pipette, then letting it fall over the surface of the filter. In the exception mentioned, the filter was covered with a rubber tube which extended one-half to three-quarters of an inch above the height of the filter, the serum being fed into the cup made by the projection of the tube. In this particular instance the attempt was made to filter the serum undiluted. After a time, however, the filtration proceeded so slowly that salt solution was added. In the remaining experiments an equal quantity of salt solution was added to the serum before filtration was begun, and in all cases the filter has been washed out with several cubic centimeters of salt solution after the serum had passed through. The serum of infected monkeys has been used throughout for filtration experiments, the serum being obtained by defibrination and centrifugation. In all instances the infectiousness of the unfiltered serum has been determined by control experiments and the serum has been used as soon as possible after obtaining it.

In no instance has infected serum even in quantities of 6 to 12 c.c. been infectious for the guinea-pig after being filtered in the manner described, although 0.5 c.c. of fresh defibrinated blood have not failed to cause the disease (see Table 9, as an illustration).

TABLE 9.
FILTRATION EXPERIMENT. SERUM OF MONKEY XII. 5 C.C. OF THE DILUTED SERUM WERE INJECTED INTRAPERITONEALLY AND THE REMAINING PORTION SUBCUTANEOUSLY.

| Guinea-Pig No. | Volume of Serum | Volume after Dilution | Incubation Period | Result | Autopsy |
|-----------------|-----------------|-----------------------|-------------------|-------------------|---------|
| Filtered. . . . | 7 c.c. | 9-10 c.c. | 7 days | Not sick Death | Typical |
| Unfiltered.. | 7 | 9-10 | | | |

In view of the possibility that minute forms of the organism, capable of passing through the Berkefeld filter, might exist within the

erythrocytes or leucocytes, whereas only larger forms might be extracellular, an attempt was made to free such hypothetical minute forms by crushing the blood cells in a porcelain ball-mill. Inasmuch as the result of this experiment points to the existence of a toxic substance in infected blood, the details may be given.

Defibrinated blood from monkey No. XIV was washed 10 times with sterile physiologic salt solution in order to get rid of the serum. This washed blood proved to be infectious for guinea-pigs in doses of 0.7 and 1.5 c.c. (see Table 3).

The washed cells from 25.2 c.c. of blood were ground in the ball-mill for six hours. The mass was removed from the mill by fractional washing with salt solution, centrifugated to get rid of porcelain sand, the latter then being washed fractionally, and the total volume of fluid being made up to 50.4 c.c. by additional salt solution. The fluid was dark red in color, cloudy, and no cells could be recognized by microscopic examination. Some of this fluid, representing 50 per cent of blood in volume, was injected into guinea-pigs in doses of 1.4, 2.0, 5.0, and 10.0 c.c., the effects of which are shown in Table 10.

TABLE 10.

TEST OF THE INFECTIONOUSNESS OF THE WASHED AND GROUND-UP CORPUSCLES OF MONKEY XIV.

| Guinea-Pig No. | Volume Injected | Equivalent in Normal Blood Volume Less Estimated 10% Loss | Result |
|----------------|-----------------|---|---|
| 170..... | 1.4 c.c. | 0.63 c.c. | Slight febrile reaction beginning on the second day after inoculation. Recovery |
| 171..... | 2.0 | 0.9 | Moderate febrile reaction beginning the first day after inoculation. Recovery |
| 172..... | 5.0 | 2.25 | Moderate febrile reaction beginning the first day after inoculation. The primary temperature subsided, but in 6 days a course of fever developed which persisted for 9 days. Recovery |
| 173..... | 10.0 | 4.5 | Similar to that of 172, but died on the 27th day after inoculation. Death due to extraneous infection, as shown by culture |

The remaining portion was passed through a fresh Berkefeld filter about three hours being occupied in filtration. Filtration proceeded rapidly at first, but more slowly later, as the fluid became more concentrated in insoluble albuminous particles. The filtrate was dark red and perfectly clear. The entire amount was injected intraperitoneally into two guinea-pigs at interrupted periods. (Table 11.)

TABLE 11.

SHOWING THE TOXIC EFFECTS OF THE FILTRATE OF WASHED AND GROUND-UP CORPUSCLES FROM THE BLOOD OF MONKEY XIV.

| Guinea-Pig No. | Volume Injected Fractionally | Equivalent in Normal Blood Volume Less Estimated 15% Loss | Result |
|----------------|------------------------------|---|---|
| 174..... | 15 c.c. | 6.4 c.c. | Death in 4 days following febrile reaction. Blood sterile |
| 175..... | 20 | 8.5 | Death in 6 days following febrile reaction. Blood sterile |

In comparing Tables 3, 10, and 11, which represent experiments performed with the same blood, one gains the impression, first, that grinding the blood in the mill had almost entirely destroyed its infectiousness, and second, that with the destruction of the infectiousness of the blood the latter manifested a rather pronounced toxicity.

In support of the first point, it may be noted in Table 3 that the blood before it was ground up produced typical infections in doses of 0.7 c.c. and 1.5 c.c., whereas after it was ground up, its infectiousness had been largely destroyed, as seen in Table 10. This effect, possibly, is due to an actual crushing of the organism in the mill.

In support of the second point, we have to note first the primary fever which developed in the guinea-pigs of Table 10, and second, the toxicity of the filtrate as shown in Table 11. In order to determine whether the intoxication seen in the animals of Table 11 was due to microbic poison or to the action of the proteids of monkey corpuscles on the guinea-pig, 12 c.c. of normal monkey blood were ground up and a filtrate prepared analogous to that injected into guinea-pigs Nos. 174 and 175. The total filtrate, 20 c.c., was injected into a single guinea-pig which had the weight of the former animals. A slight rise of temperature which occurred on the fifth day after injection lasted two days; otherwise there was no disturbance. It may accordingly be concluded that the intoxication of animals Nos. 174 and 175 was not due to the normal proteids of the corpuscles of monkey's blood. One could scarcely consider the condition an infection since the same blood before filtration had shown practically no infectious properties (Table 10). It could hardly be due to soluble toxins in the monkey's blood, since such toxins probably would have been removed by the washings to which the blood had been subjected.

Although further experiments are indicated before positive conclusions are drawn, it seems probable that the intoxication of the animals was due to the liberation of poisons by the crushing of the virus in the ball-mill.

RESISTANCE TO HEAT.

Heating experiments have been performed at temperatures of 45° and 50° C. It was necessary to use rather large quantities of blood in order to be certain that a fatal quantity of organisms was being dealt with. On this account it was preferable to use relatively low temperatures over rather long exposures in order to insure as complete diffusion of the heat as possible.

Results.—In an experiment in which 3.5 c.c. of blood were heated at 45° C. for 5, 10, 15, 20, 25, and 30 minutes, all animals died of spotted fever. In two experiments in which the blood was heated at 50° C., the infectiousness of the virus was destroyed in 25 minutes in one series and in 30 minutes in another.

RESISTANCE TO DESICCATION.

The following is the technique used in desiccation experiments: Uniform quantities of blood are distributed into open Petrie dishes which are placed in a desiccator over sulphuric acid and dried as quickly as possible under vacuum at room temperature. Desiccation requires from 18 to 24 hours, depending on the degree of exhaustion of the bell-jar. When desiccation is complete the plates are placed in an ordinary sulphuric-acid desiccator in the ice-chest.

Results.—Two series of experiments showed the loss of pathogenicity at some time between 24 and 48 hours after complete desiccation.

VIABILITY IN THE ICE-CHEST.

The M. L. D. of the blood of Monkey No. XV, which when drawn was about 0.1 c.c., had increased noticeably in five days, reached 2.0 c.c. in 11 days, and in 15 days 3.0 c.c. failed to produce infection. The blood was kept in the ice compartment of the ice-chest. In another instance 5.0 c.c. retained infectiousness for 16 days.

TRANSMISSION BY MEANS OF THE MALE TICK

The possibility of transmission by means of the male tick (*Dermacentor occidentalis*) has been demonstrated conclusively in a recent experiment. The tick was one which had been raised from the egg in the laboratory, the life-history of the brood having been published previously (*loc. cit.*).

Infection of the tick was accomplished by feeding on two sick guinea-pigs in the following way: On October 16 it was placed on the ear of guinea-pig No. 107, where it remained for about 12 hours at the end of which time the guinea-pig died. Two days later it was placed on the ear of guinea-pig No. 121, where it remained for about 20 hours, or until the guinea-pig died. After an intermission of three days the tick was placed on the ear of a healthy guinea-pig (No. 169), and the latter died in 13 days showing changes which have been recognized as characteristic of spotted fever. However, since an adventitious epidemic had developed among the guinea-pigs and since areas of focal necrosis found in the spleen of guinea-pig No. 169 were not entirely typical for spotted fever in the guinea-pig, the experiment was not considered conclusive and the animal was discarded. On November 7, 17 days after the tick had been removed from the infected guinea-pig (No. 121) it was again placed on a healthy guinea-pig (No. 182). It was allowed to remain attached for $3\frac{1}{2}$ days, after which it was removed. Ten days after the tick was placed on the guinea-pig the latter suddenly developed high fever and died in five days, showing those anatomical changes which have proved to be diagnostic of experimental spotted fever.

Autopsy.—Scrotum moderately hemorrhagic and very cyanotic. Hemorrhagic condition is seen best by the naked eye in cutting through the skin. Testicular coverings are moderately congested and the anterior pole of the testicles deeply infiltrated with blood. The axillary, inguinal and mesenteric lymph glands are enlarged and hemorrhagic. The spleen is several times the mass of the normal spleen and cyanotic in color. The kidneys are congested and cyanotic; suprarenal glands are enlarged; liver enlarged, congested and cyanotic. The lungs and heart show no appreciable changes. The meninges are slightly reddened. At the point of the tick bite is a necrotic crusted wound about one-fourth inch in diameter. Cultures from the heart and peritoneum yielded no growth.

From the organs of this guinea-pig inoculations were made into two other guinea-pigs, one of which died in seven and the other in

eight days, both showing typical anatomical and clinical phenomena of spotted fever. Monkey No. 17 which was also inoculated from guinea-pig No. 182 ran a typical course and presented extensive scrotal hemorrhage.

From the second generation in guinea-pigs and also from the monkey, inoculations were made into a third generation, the members of which ran typical courses. This is being continued by successive inoculations of the guinea-pig.

The experiment is regarded as conclusive.

CONTINUOUS PASSAGE THROUGH THE GUINEA-PIG.

In a previous article (*loc. cit.*) my failure to keep spotted fever alive by the successive inoculation of guinea-pigs was referred to. In the earlier attempts fresh inoculations were made only as the guinea-pigs were about to die or after they had died. The possibility was recognized that the quantity of living virus in an infected animal may be greater early in the course of the disease than at the time of death, hence at a convenient time the attempt was made to perpetuate the infection in the guinea-pig alone by inoculation with blood or organs taken on the third to the fifth day after fever had begun. This method has proved entirely successful through five and into six generations of guinea-pigs. Hence it seems probable that the monkey can be dispensed with for the purpose of maintaining the disease in the laboratory.

SUMMARY.

Rocky Mountain spotted fever is transmissible to the guinea-pig and monkey by the inoculation of defibrinated blood of patients suffering from the disease.

The virus may be kept alive in the laboratory either by alternate inoculation of monkey and guinea-pig, or by continuous passage through the guinea-pig by observing the method described above.

The disease is transmissible from one animal to another by means of the bite of either the male or female tick (*Dermacentor occidentalis*).

One attack of the disease establishes a rather high degree of immunity to subsequent inoculation.

Attempts to pass the virus through Berkefeld filters have failed.

The parasites are not located essentially in either erythrocytes or leucocytes but are present in the body fluids generally.

By grinding infected blood in the ball-mill infectiousness is largely destroyed; in this process there is some reason to think that the organisms are crushed and that toxic substances are thereby liberated.